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A MICROSCOPE LAMP ESPECIALLY DESIGNED FOR THE BELLING METHOD[1]

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ABSTRACT.—The special possibilities of the illuminant proposed depend on the presence of two filaments, which can be operated either singly or both at once and are so small and close together that they form a concentrated source even when both are in use; and on the special adaptability of the headlight bulb for overloading. It is shown that within the range of from 6 to 8 volts per filament for which the bulb is made, the two filaments when run together yield a maximum of about 160 C. P.; while the 32 C. P. of single filament at its design voltage provides a reduction to 1/5 of the maximum without reducing the color-temperature below that required for the correct performance of "daylight" filters. The higher intensities, with proper concentration, permit the use of very dense green filter combinations.

The bulb is small enough to permit a very effective reflector, making condensing lenses unnecessary. It develops relatively little heat. It is immediately replaceable almost anywhere. Ordinary small transformers provide the necessary voltage range.

The lamp described conforms to the requirements of the Belling method, but includes some new means for putting these principles into practice. The headlight bulb is substituted for low-voltage projection bulbs; the thin metal diaphragms are replaced by a thick brass slide, in which the optical effect of extreme thinness is obtained by beveling the openings to a sharp edge; and a "hood-tube," projecting from 15 to 20 cm. from the plane of the diaphragmed ground-glass, combines the two functions of eye protection and filter support, confining all unfiltered light, and placing the filter in a protected position inside the end cap of the added tube. Constructional detail is given in the description, supplemented by a drawing and a photograph.

[1]This lamp was shown and briefly described in a talk on some experiments with Belling's methods, presented at a meeting of the Society of Hygiene of the Johns Hopkins University, Baltimore, April 11, 1934.

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The Proposed Illuminant

In seeking a source of light suitable for use in a lamp to be designed in accordance with Belling's principles, the writer was impressed by certain apparently unique possibilities of the common 32, 32 C. P., two-filament headlight bulb, "Mazda No. 1000." Altho theoretically of a rather low spherical candlepower, Mazda No. 1000 has a number of special characteristics which should, it seemed, more than offset this apparent disadvantage. Preliminary experiments confirmed this belief, and led to the construction of the microscope lamp described in the second part of this paper. Used in such a lamp, the proposed illuminant has given the writer a more satisfactory illumination for the microscope than he has obtained from any other incandescent source—one of the most striking advantages being the possibility of obtaining from the one source of light both a graduated series of high intensities, great enough for use with green filters of narrow spectral range, and also a low-intensity light *of normal color-temperature*,[3] such as is required for the correct performance of an "artificial daylight" filter. Such a color-temperature at a greatly lowered intensity obviously cannot be obtained from an ordinary high-intensity source merely by operating it at a subnormal voltage, since the color-temperature is reduced with the intensity.

This unusual range of intensities is the combined result of three special characteristics. First, the two filaments, tho intended for alternate use, can readily be connected for the operation at will, either of a single filament or of both at once; and the two filaments constitute a concentrated source even when both are in use. Second, filaments for automobile use are, of necessity, designed to tolerate a circuit voltage of from 6 to 8—a range which includes a voltage overload of more than 29% (1.8 v.) over the design voltage of 6.2 (Westinghouse Lamp Co., 1932). Third, the headlight bulb in question is smaller in external dimensions than other incandescent sources of similar maximum output, and the efficiency with which its candlepower can be utilized is correspondingly greater.

An approximation of the effect of the voltage overload mentioned can be obtained from a graph of the operating characteristics of small Mazda filaments which appears in the Westinghouse booklet cited. The voltages shown extend only to 20% above the normal, and the spherical candle-power curve reaches the top of the graph (180% of

[3] That of the light emitted by a tungsten filament when operated at the efficiency for which it was designed—0.74 watts per candlepower, in the case of Mazda No. 1000. Theoretically, the daylight filter should be designed for this exact color-temperature. but, in actual practice, Wratten filter No. 78, designed for the color-temperature of the light emitted by a tungsten filament consuming 1.25 w.p.c., is quite satisfactory for white-light work with the microscope.

the normal light output) before intersecting the right-hand limit (120% of the normal voltage). The spherical C. P. curve becomes almost a straight line before reaching the top of the graph, however; and the curve was extended as a straight line to obtain the following (presumably minimal) indications: For the two filaments in series, at 14 volts (12.9% overload), 97 spherical C. P.; and at 16 volts (29% overload), 147 spherical C. P. A very simple form of the Ritchie photometric wedge method gave, as would be expected, somewhat higher values: at 12 volts, 68 C. P.; at 14 volts, 102 C. P.; and at 16 volts, 163 C. P. The high value obtained at 12 volts is probably due largely to the mutual heating effect, the two filaments being only 0.14 in. apart.

The gain in efficiency made possible by the small size of the headlight bulb is in part evident in the drawing. In the first place, the maximum diameter of the headlight bulb being only 1.25 in., the ground-glass can be brought much closer to the center of light than is possible in the case of most bulbs of comparable candlepower; and the intensity, per candlepower emitted, of the illumination of a small area surrounding the point of contact of the ground-glass with the wall of the bulb is greatly increased. Differences in the form and size of the filaments preclude a direct comparison on the basis of the law of inverse squares, but in most instances the headlight bulb has the advantage of this difference also, its light source being the more concentrated. Second, the miniature over-all dimensions of Mazda No. 1000 permit a more efficient reflector than is practicable with larger bulbs. Since the diameter and depth of the reflector must be increased to maintain the angular magnitude of the recoverable cone of the back light (about in proportion to the largest dimension of the bulb used) large bulbs would seem to demand either very bulky apparatus or a serious sacrifice of reflector efficiency.

High intensity is attained in some well-known lamps, of course, by using reflectors of small efficiency with special projection bulbs giving a very large output of light (and heat); and adding a pair of condensing lenses (which add their focal distance to the necessary length of the lamp, and do not conserve as large a proportion of the total light output as does a good reflector). To the writer, however, both the heat and the lenses seem unnecessary evils in a microscope lamp—unless a *corrected* condensing lens forms a part of the optical system, as in Köhler's method.

Small tubular projection bulbs (50 watt, about 64 C. P.; 100 watt, about 151 C. P.) have filament dimensions and bulb diameters similar to those of the headlight bulb; but they have only a single fila-

ment. They are rather delicate and must be burned within 25° of vertically, base down; they are longer than the headlight bulb; and they do not compare favorably with it in certain other respects noted below.

Desirable features of the headlight bulb other than those already pointed out may be summarized with little comment. The heat output is relatively low. This fact, with the miniature size of the bulb, permits a compact, nearly light-tight housing. In contrast with projection bulbs and other special-purpose bulbs of high wattage, Mazda No. 1000 is not only cheap but is replaceable at a moment's notice almost anywhere. Unlike low-voltage bulbs consuming 100 watts or more, the headlight bulb does not require a specially made step-down transformer. A "toy train" transformer of 100 watts rated capacity—exceptionally convenient for the purpose because of its five-point switch which gives each pair of taps a 10-volt range—meets the amperage requirements, if the two filaments are connected *in series* when operated together. Only two pairs of taps are needed to cover more than the voltage range used.

The working life of the headlight bulb as used in the microscope lamp cannot at this time be stated with any exactness. In the lighting service for which it was designed, its average life is given as 100 hours (as against 50 hours for projection bulbs). The one bulb of which the writer has kept a full record has thus far given somewhat more than 30 hours of service with both filaments lighted, and shows only a very slight darkening of its walls.

In comparison with the mercury-vapor arc, the proposed illuminant is considerably more convenient to use; it provides white light without the necessity for turning to another source; and, as used in the lamp here described, it permits the use of filters passing narrow spectral bands which seem to give all the advantages of improved definition and resolution which can be gained by using the pure monochromatic green light obtainable from the mercury-vapor source. It would seem that there would probably be some difficulty, too, in concentrating at all efficiently the light of a tubular source upon a small circular area.

The Construction of a Microscope Lamp for the Belling Method

The writer has departed in no way from the principles indicated by Belling. He has, however, made certain changes in the means adopted for putting these principles into practice. The modifications affecting the design of the lamp are as follows:

1. The use of Mazda No. 1000 in place of the 108-watt, 6-volt projection bulb—a change fully discussed in the preceding section.

2. The use of a reflector in place of condensing lenses. The writer's preference for a reflector, in the case of any illuminant small enough to permit the use of an efficient one, has also been dealt with.

3. Belling (1930) mentions diaphragms "of blackened card or thin metal" (p. 67), and at several points refers to "thin metal" diaphragms. The metal must evidently be *very* thin, if one is to avoid marginal rings of partial shadow—these penumbral rings being especially annoying when the lighted area is to be limited to a small circle in the middle of the field. Since a diaphragm thin enough to render this defect negligible would be easily damaged, the writer prefers to drill the diaphragm openings in a *thick* ($\frac{1}{16}$ in.) brass slide, and to gain the optical effect of extreme thinness by beveling each opening to as thin an edge as possible.

4. Belling supports the filter before the mirror (or prism) of the microscope, and adds a separate arrangement—an opaque screen before the eyepiece—to protect the eyes. The writer prefers to attach neither of these arrangements to the microscope, but to combine them in the form of the "hood-tube," which he has made an integral part of the lamp itself. By this means the glare of the unfiltered light is prevented from escaping into the room in any direction. This is especially important in the use of the denser filters, which require the incident light to be so intense that its reflection from the polished parts about the base of the microscope is a serious inconvenience. The hood-tube, or some equivalent device to confine all unfiltered light, becomes a practical necessity in the case of such filters as the yellow-green and the blue-green recommended for use with the lamp here described.

The filter holder inside the cap of the hood-tube insures, if the cap be made with reasonable accuracy, that the filter will always be placed in the correct position with respect to the illuminating beam; and it protects the filter from displacement during use, from mechanical damage, and, to some degree, from direct daylight.

5. "The yellow-green Wratten series, Nos. 66, 56, 57A, 58, and 61, affords a graduated sequence of amount of absorption; and the members can be used.....for regulating the intensity of the light" (Belling, 1930, p. 75). To this method of controlling intensity the writer suggests an objection based upon one of the four primary rules of modern microscopy emphasized by Belling himself: namely, that "the light should approximate to yellow-green monochromatic whenever possible." If such a series of filters be the means of controlling

the intensity, the more light there is needed, the less closely can the light "approximate to yellow-green monochromatic." Moreover, as the writer has already pointed out (Waterman, 1934) filters of a transmission which approximates yellow-green (or bluish-green) monochromatic much more closely than do any of those of the series named can readily be used; they also carry Belling's principle nearer its logical limit, and, in actual practice, they produce a distinctly greater improvement in sharpness and resolution. The writer, therefore, prefers to do all green-light work with only those filters which give the best images, and to obtain all needed adjustment of intensity by varying the intensity of the illumination of the ground-glass itself. The exceptional adaptability of the headlight bulb to such a purpose has already been discussed.

CONSTRUCTIONAL DETAILS

These have been indicated as fully as seemed practicable in the drawing, and the viewpoint for the photograph has been chosen to show parts not readily represented in section and elevation. Only points which it was not found possible to make plain in the illustrations will be taken up here.

The *housing*, including the ventilator hoods, the top sector, (T-S) and the small added casing which contains the toggle switch, are of light sheet metal, "tin" (tinned sheet iron) being quite satisfactory (especially with respect to the ease with which it can be soldered neatly). The three wiring protectors (W-P) are short pieces of copper or brass tubing, their edges well rounded in a lathe, which are to be soldered thru the openings carrying the wiring to prevent cutting of insulation.

Ventilator openings of the form and size specified are adequate to keep the housing from becoming warm enough to render the enamel sticky; and the *ventilator hoods*, soldered light tight to the housing, and, at the front, to the top sector above and to separate end pieces below (so that they open only to the rear of the lamp at the points marked "inlet" and "outlet") allow but very little light to escape in any direction.

The *slot frame*, about the opening thru which the adjusting knob (K), is attached to the slide (R-S) upon which the reflector is mounted, is required to prevent K from rubbing and sticking on the enamel of the housing. Its opening is $\frac{1}{8}$ in. wide, and its length should be sufficient to allow the reflector a total excursion of about 1 inch. Both to give the needed strength and for neatness of appearance of the work, the pieces which are to form this frame should be soldered to the housing by a blowpipe flame, directed upon the brass, rather than with a soldering copper; since the last-named tool is not likely to flow the solder fully and freely under the brass pieces (Newman and Clay, 1933).

The nature of the *reflector slide* would seem to be sufficiently indicated. Attention may be drawn, however, to the fact that it must move between guides adequate to prevent any such play as would displace the focal spot of the reflector from the center of the ground-glass.

The *toggle switch* is of the type used in radio sets. It should be capable of carrying about 4 or 5 amperes.

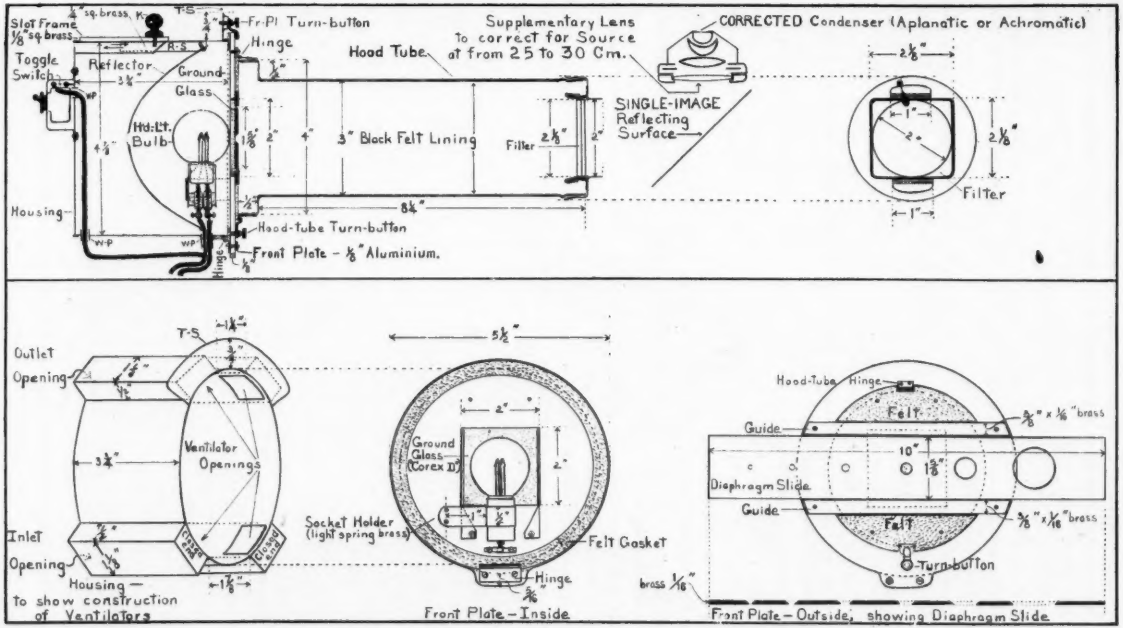

FIG. 1. MICROSCOPE LAMP FOR BELLING'S METHOD. DIAGRAM

The single *supporting trunnion*, shown only in the photograph, is made by soldering a ½-inch shaft collar to the housing, and setting in this collar, by means of its set screw, a piece of ½-inch steel shafting about 1½ inches long and finished to a rounded end in the lathe. The soldering of this collar to the side of the housing *must* be done by heating the heavier piece of metal with the direct flame, if the joint is to have strength enough to support the lamp. The writer "tinned" the under surface of the collar thoroly with a copper, in addition to placing solder and soldering paste both inside and outside the collar before heating it.

Theoretically, the most efficient form for the *reflector* would evidently be an ellipsoidal one, the light center being placed at one of the foci of such an ellipsoid as would have its other focus at the point of contact of the wall of the bulb with the ground-glass. Because of the short distance (⅝ in.) between the foci, however, in the case of the headlight bulb, and the concomitantly very slight departure of the ellipsoid from the form of a true sphere, a hemispherical mirror would have very nearly the same actual effectiveness—especially so, since the ellipsoidal mirror could not be brought to true focus without forming an objectionably sharp image of the filaments on the ground-glass. The hemispherical mirror could be cut from a blown glass bulb about 4 inches in diameter, silvered on the back, and mounted in a light metal frame protecting it from breakage.

For the practical purposes of the microscope worker who wishes to make up a lamp for himself, however, such an approximately *paraboloid* reflector as that indicated in the drawing—a light, silvered-metal mirror which can be bought ready-made in the form of a cheap automobile "spotlight"[4]—is very effective and is recommended as giving as efficient a concentration as the writer has found necessary.

The *front plate* is made from ⅛ in. aluminium, to combine lightness with the rigidity and reasonably accurate planeness of surface which is necessary to permit the diaphragm slide to fit close against the front plate. The form required is easily cut out, either with a small power jig saw or with a jig saw blade in a hand frame. The edge is most readily finished with a small sanding drum, but a satisfactory finish can be had with hand tools.

The *socket* for the headlight bulb is of the standard two-contact "adjustable" type used in automobile head lamps; but that part of the socket intended to project thru the back of the head lamp reflector, and to receive a plug connected to the feed wires, must be sawed off, as indicated in the drawing, close to the insulating material in which the contact pins are embedded. Short pieces of thick-walled brass tubing, ⅛ in. outside diameter, are then drilled and tapped for No. 2–56 set screws, and are soldered with their ends against the sides of the contact pins in the manner indicated, care being taken that the solder also attaches the pins firmly to the spring tubes from which they project, preventing the pins from turning.

In the section of the entire lamp, the set screws are represented as projecting in *opposite* directions, whereas in the view of the inside of the front plate both screw heads project toward the rear of the lamp. The latter is obviously the correct arrangement; but only one of the screws could have been shown, in the correct position, in the section view.

The ends of the added wire tubes should be tinned before applying them to the contact pins, to minimize the heat carried by the pins into the insulating material. In the writer's lamp the altered socket was soldered into the spring-brass socket holder. With care not to make contact with either of the two contact pin tubes embedded in the block of insulation, the metal shell of the socket could be drilled and tapped for two or three No. 2–56 (or smaller) screws, and could then be attached to the spring

[4]Spot-light No. 25-A, by the Pines Winterfront Co., Chicago, Ill.

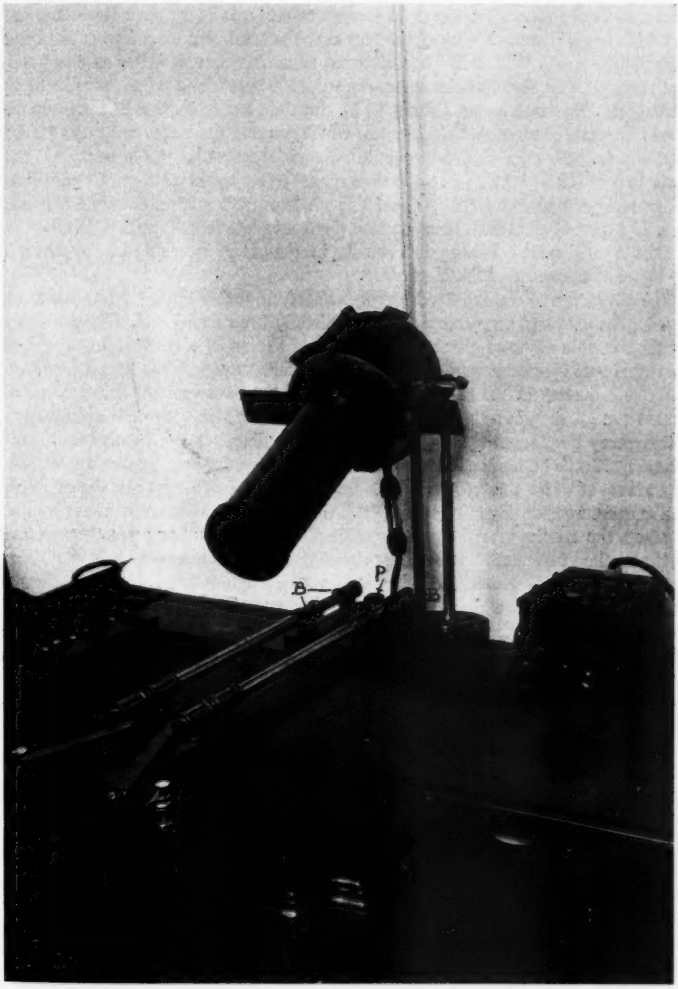

FIG. 2. MICROSCOPE LAMP FOR BELLING'S METHOD PHOTOGRAPH

holder more neatly and with less heating of the insulation. In either case the socket must be carefully placed so that the bulb inserted in it will be in the position fully exposing both filaments toward the ground-glass.

The *ground-glass* must be of a heat-resistant type, the local concentration in the focal spot of the reflector being great enough to crack ordinary glass (including lantern slide cover glass), in spite of the fact that the total heat evolution of the headlight bulb is small. "Corex D" has been recommended[5] to the writer as adequately heat resistant, and slightly more transparent than "Pyrex"; and it has proved entirely satisfactory. Two-inch squares about $\frac{1}{8}$ in. thick are available. One of these squares should be *finely* ground on *both* sides, for which purpose the writer used first Carborundum No. 100, then Carborundum FRA, each abrasive having been mixed with water to form a fairly thick paste and then spread on a piece of plate glass, upon which the grinding can be conveniently done. The whole operation requires only a few minutes. The ground-glass is to be fitted into the front plate loosely enough to allow for a slight expansion. It is held against the diaphragm guides by spring fingers shown in the figure of the inside of the front plate.

The advantage of making the *diaphragm slide* from rather thick ($\frac{1}{16}$ in.) brass, and beveling its openings to a sharp edge, has already been mentioned. The size of the openings can be adjusted closely to the needs of the set of objectives and eyepieces most used for high-power work by first focusing the ground-glass, with all conditions correctly adjusted, in the object plane, and marking on a piece of thin paper held against the ground-glass the diameters of the circles visible with each combination. A temporary diaphragm opening about $\frac{1}{8}$ inch in diameter will be needed, in conjunction with the oil immersion $\times$ 90 to $\times$ 100 objective, for determining the distance of the lamp at which the "ring-test" (Belling, 1930, p. 88) is absent and the entire objective aperture is evenly filled with light. With the correct distance of the lamp thus obtained, the openings for the permanent diaphragm slide will be correctly measured by the method indicated. The smallest opening should illuminate a circle of a diameter about $\frac{1}{3}$ that of the field of the high-power oil immersion, if the corrections of the condenser are good enough so that it will illuminate the full N. A. of the objective from a source of this size (Belling, 1930, p. 67). The opening required will be in the neighborhood of 1 mm.

If of brass, as recommended, the diaphragm slide and its guides can be given a firmly adherent and heat-resistant black finish by immersing them for a few minutes in a mixture of 40 cc. of strong ammonia and 100 cc. of distilled water, *saturated* with copper carbonate. The copper carbonate must always be present in excess. The brass must be absolutely free from grease or oil of any sort, and should not be touched with the fingers between cleaning and immersion in the ammoniacal copper solution.

The *hood-tube*, like the housing, is of "tin." Details not clearly shown in the drawing are a binding of felt, turned over the edge of the rimmed flange; a felt covering, which can be seen in the photograph, on the outside of the cap; and the manner of joining to the top of the rimmed flange the hinge thru which it is attached to the front plate. The hinge is soldered to the outside of the rimmed flange, the joint being concealed under the felt binding, and the remaining leaf of the hinge is attached to the front plate by the small screws shown. The felt binding of the rimmed flange is pressed against the felt-covered segments on the front plate, when the hood-tube is locked in position by the turn button below, to form a practically light-tight joint. This binding is also needed to avoid the scratching of the blackened surface of the diaphragm slide. The felt covering of the outside of the filter-holder cap is necessary because an

[5]By the Corning Glass Works, Corning, New York, makers of both of the glasses named.

enamel finish on this cap is quickly rendered unsightly by frequent handling. Thick shellac, allowed to become very sticky before the felt is applied, is a satisfactory adhesive. A detail not shown in the drawing is a half-turn screw-thread, pressed into the end of the hood-tube and its cap.

The importance of the *black felt lining* of the hood-tube can hardly be overemphasized.

For many modern microscopes (especially such instruments as the "DDE" binocular, with reversed stand, of Bausch and Lomb), the hood-tube as shown in the drawing and photograph will be found too long to permit the correct adjustment of the distance of the lamp. As indicated in the following paragraph "*Ready-made Parts*. . . . ." the tube proper, dimensioned in the drawing as 8¼ in. long, can just as conveniently be made 5¾ in. long. It is recommended that the hood-tube be made in this shorter form, *not* in that shown. The headlight bulb, as used in the lamp here described, does not appreciably warm filters placed 6 in. from the ground-glass.

The *wiring* of the lamp needs little comment. It is so connected that if the toggle switch be closed the current passes thru the walls of the housing, the front plate, etc., to the outer shell of the base of the bulb, lighting only the front filament; but with the toggle switch open, both filaments are lighted in series. The photograph indicates the manner in which the writer effects the change from one pair of transformer taps to the other. Two-contact sockets and a corresponding plug of the automobile type would be much better than the line-voltage outlets and plug head shown, in that the automobile type of plug cannot be inserted accidentally into a line-voltage outlet.

The *screw sizes* require no rigid specification, except that the front plate, only ⅛ in. thick, probably should not be tapped for any thread coarser than 32 to the inch. In the writer's lamp, the screw set in K and turning in R-S, the 2 screws attaching the front plate to its hinge, and the two which attach the casing of the toggle switch to the housing, are No. 4–36. The screw in K is iron, the others, round-head brass. Two round-head brass screws, No. 3–48, attach the spring socket holder to the front plate, and are cut off flush with the outer surface. The two screws holding the hood-tube hinge to the front plate, and the four which attach the felt-covered segments are also No. 3–48. The hinge screws and the two upper segment screws are cut and ground flush inside, but the two lower segment screws project about ⅛ in. inside, thru the spring fingers which keep the ground-glass in place. The fingers are held by hexagonal nuts and washers. The two diaphragm guides are attached with four round-head brass screws, No. 2–56. The two *turn buttons* are No. 8–32, knurled-head, brass screws, to which brass tongues of the form indicated have been soldered. The front plate turn-button turns, as shown, in a hexagonal nut soldered to the top sector, T-S, while the hood-tube turn button is provided with a thread tapped in the front plate.

A satisfactory *finish for the sheet metal parts* is obtained by cleaning with benzol and alcohol, applying a single coat of a metal primer, smoothing lightly with very fine sandpaper (5/0 to 7/0) and adding two coats of a "four-hour" enamel, the first being smoothed like the primer, the second by rubbing lightly with pumice powder and linseed oil. "Four-hour" enamels seem to take about 24 hours to dry enough for rubbing.

Ready-made Parts for Housing and Hood-tube. It is perhaps evident in the photograph of the completed lamp that the writer used certain standard forms of "tin can" as the basis both for the housing and for the hood-tube. This was not done to save either cost or labor, but because such details as the circular seam at the back, the turned, reinforced edge at the front of the housing, the rimmed flange, the pressed half-turn cap-thread, etc., of the hood-tube are practically impossible to make as neatly by hand or in the ordinary machine shop as they are made, with specialized machin-

ery, by the can manufacturer. Coffee, tobacco, etc., are "vacuum-sealed" in a stout tin of the dimensions given for the housing. The tin needed for the hood-tube is used for cookies of several makes. For the shorter form of hood-tube, the tin, 3 inches in diameter and 5¾ in. long, with half-turn cap, which is used for Merck's *p*-dichlorbenzol ("Dichloricide") is excellent. The rimmed flange is made from a pressed (one piece) slip-on lid, ½ in. deep, and 4 in. in diameter. These parts are recommended as likely to give the completed lamp a better appearance than corresponding forms made up out of sheet metal, either by the microscopist himself or by a tinsmith under his direction.

In joining the hood-tube proper to its rimmed flange, distortion of either part can be avoided by centering the 3 in. tin on the 4 in. lid, and soldering the entire joint smoothly and firmly *before* cutting out either the bottom of the tin or the corresponding part of the lid. The opening can then be cut out with a stout knife, close to the seam, and quickly finished with a rasp and sanding drum.

SUGGESTED MODIFICATIONS OF THE DESIGN

With the bulb in a fixed position, close against the ground-glass, only the small areas used in rather high-power work can be evenly illuminated. To remedy this, both bulb and reflector can be mounted on a slide, moved by rack and pinion, the reflector being focussed on the point in the wall of the bulb which will touch the ground-glass when the slide is racked up close. The writer, however, merely withdraws the diaphragm slide altogether for low-power work and adds a fitted circle of ground-glass, placed in the rimmed flange of the hood-tube. This additional ground-glass may be provided with a black paper diaphragm, attached with shellac. Since the 16 mm. objective and lower powers do not need very accurate conditions of illumination for most work done with them, the ½ in. alteration in lamp-distance usually need not be corrected.

If a tin is *not* to be used as the basis for the housing, it is suggested that the housing be made of square cross section, this form being easier to make of satisfactory appearance than is the cylindrical.

Filters, Mirror, Condenser, etc. Belling found the aplanatic condenser (spherically, but not chromatically, corrected) to be "nearly equal to the achromatic-aplanatic condenser, both being used with a rather dense yellow-green screen, such as Wratten No. 58" (Belling, 1930, p. 85). As indicated in Table 1, the much more nearly monochromatic filter combinations, Wratten No. 15 + No. 45, and No. 47 + No. 61, can be used for all green-light work at the "maximum useful magnification" (1000 × working N. A.), or a little above: and with either of these combinations the performance of the aplanatic condenser cannot be distinguished from that of the achromatic-aplanatic. The latter will only be needed if some of the critical work must be done with white light. The *uncorrected* condenser (the so-

called "Abbé") is, of course, useless for any small-source illumination method.

The common form of plane substage mirror largely defeats the purpose of the diaphragming of the light-source, and should not be used (see photomicrographs, preceding article, p. 99). Either the reflecting prism (Belling, 1930) or the lacquer-protected, front-silvered

TABLE 1.—APPROXIMATE SPHERICAL CANDLEPOWERS OF MAZDA NO. 1000 WITHIN THE RANGE, 6 TO 8 VOLTS PER FILAMENT (12 TO 16 V. WHEN THE 2 FILAMENTS ARE USED IN SERIES); AND ILLUMINATION INTENSITIES GIVEN BY THE PROPOSED LAMP WITHIN THIS RANGE, WITH VARIOUS FILTERS

Volts	Voltage overload, *per cent*	Spherical candle powers, *approximate measurement*	Intensities given by proposed lamp with various light filters			
			Daylight filter (Wratten No. 78)		Wratten green filters	
			Color sensation	*Intensity*	*Filter*	*Intensity*
6.2 (SINGLE filament)	None	32 (Design C. P.)	Daylight	2	No. 15 + No. 45 No. 47 + No. 61	—1 —2
12 (BOTH filaments, *in series*)	None	68	Daylight	3 to 4	No. 15 + No. 45 No. 47 + No. 61	2 1 to 2
14 (BOTH filaments, *in series*)	12.9	102	Bluish	4+	No. 15 + No. 45 No. 47 + No. 61	2 to 3 2
16 (BOTH filaments *in series*)	29.0	168	Strongly bluish; intolerable intensity		No. 15 + No. 45 No. 47 + No. 61	3 to 4 3

Scale of Intensity Estimates: —2, dim. —1, still inadequate. 1, adequate for lightly stained preparations. 2, adequate for preparations of ordinary density. 3, adequate for over-stained parts of sections, but rather strong for ordinary stainings unless illuminated area is made very small by source diaphragm. 4, too strong for continuous bright-field work on ordinary preparations.

Conditions of the Experiment: Zeiss aplanatic condenser, with supplementary lens for near source and water immersion; lamp-distance adjusted by "ring-test"; front-silvered substage mirror. O. I. objective, N. A. 1.3, × 95, condenser open to about N. A. 1.2; Hyperplane eyepiece, × 15; all other conditions adjusted for sharp image (with the green filters), free from light fog. Total magnification, × 1425, or working N. A. × 1140. Monocular microscope. Light enough for the two green filters can also be had in the case of the binocular instrument.

mirror described by the writer (1935), enables the condenser to form a sharp, single image of the light-source which can be focused accurately in the plane of the object.

The two Wratten combinations mentioned above are the only green filters needed with the lamp here described. The Wratten filter No. 78 provides an excellent white light for work in which

color distinctions are temporarily more important than the highest attainable definition and resolution.

Correct lamp-distance, as indicated by the quick and simple "ring-test" (Belling, 1930, p. 88) must be maintained at all times to avoid greatly reduced N. A. in the illuminating cone; and water (or oil) immersion of the condenser, for high-power dry objectives as well as for the water and oil immersion objectives is necessary to avoid loss of aperture and spherical aberrations. To provide a convenient adjustment of lamp-distance, the writer locks his microscope onto a small platform attached to steel bars sliding rather stiffly in the bearings marked "B" in the photograph of the lamp. The lamp-distance, once established, is not easily disturbed, tho it is readily readjusted to correct for variations in thickness of the slide; and the whole set-up, microscope and lamp together, can be swung aside on the pivot marked "P" without the loss of any adjustment.

ACKNOWLEDGMENTS

Dr. A. E. Longley and Mr. M. E. Fowler, of the Bureau of Plant Industry, enabled the writer to test the performance of the new lamp with the monobjective binocular microscopes of two makes. Dr. Longley also read the completed paper and made valuable suggestions. Mr. R. W. Trullinger, engineer, and Mr. H. P. Barss, botanist, of the Office of Experiment Stations, gave the writer the benefit of criticisms of the drawing, and of the completed paper, respectively. To these, and other members of the scientific staff of the U. S. Department of Agriculture, the writer wishes to express his thanks.

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A NEW HEMATOLOGICAL STAIN

I. Constituents and Methods of Use

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With the technical assistance of H. J. Kingsley

ABSTRACT.—**The stain proposed by the author is quickly and simply prepared by mixing equal parts of the following permanent stock solutions:**

Solution I

Methylene blue, U. S. P., med	**0.0650**	**g.**
Methylene azure A	**0.0100**	**g.**
Glycerin, C. P	**5.0**	**cc.**
CH_3OH, C. P	**5.0**	**cc.**
Distilled water	**25.0**	**cc.**
Buffer, pH 6.9	**15.0**	**cc.**

Solution II

Methylene violet (Bernthsen)	**0.0130**	**g.**
Eosin, yel., w. s	**0.0450**	**g.**
Glycerin, C. P	**5.0**	**cc.**
CH_3OH, C. P.	**10.0**	**cc.**
Acetone, C. P	**35.0**	**cc.**

The mixed stain is usable for at least eight months, and is applicable to practically all hematological purposes: blood smears, fixed sections, frozen sections, and touch preparations. In the technics utilized to produce its action on preparations treated in different ways, the only variants are the methods for treating the cells, while the stain itself remains totally unchanged for all purposes. For blood smears fixed with methyl alcohol, the technic consists merely of pouring the stain on the slide, leaving it for 5 to 7 minutes, and then washing it off. On sections, a further process of differentiation with acid acetone is rapidly carried out. The various types of granules, including megakaryocyte and platelet granules, are clearly demonstrated. For frozen sections, the technic is extremely rapid, yet yields excellent differentiation.

INTRODUCTION

The stains most used in hematology are of the Romanowsky type. They produce metachromatic effects with a characteristic reddish purple nuclear tint. The ones most commonly used are Wright's

[1]Submitted to the Graduate School of Northwestern University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

and Giemsa's for blood smears and Wright's tissue stain, Schridde's azure-II-acetone method, and Maximow's azure-II-eosin technic for sections. In a vague manner, it is generally realized that dissimilarities exist in the results obtained by these stains and technics. That specific differences are apparent between these dyes and that chemists have analyzed their composition is, however, far more poorly appreciated.

Two parallel, but practically unconnected, lines of investigation have led to present knowledge of these stains. The one, conducted by biologists, began with Romanowsky in 1890 and resulted in Wright's stain (1902). The other, contributed by pure chemists, started with Bernthsen in 1885 and merely increased chemical knowledge. Only two men have ever effected a union of chemical and biological results to solve the problem of stains for blood: Giemsa (1902 to 1904) and MacNeal (1906 to 1925). The former, however, has never published his method for preparing azure I, the most important constituent of his stain; and MacNeal's stain has not achieved the prominence it deserves.

Excellent general historical reviews of both phases of development are given by MacNeal (1906, 1925) and by Conn (1929). From the work of previous investigators and from my own experiments, it is obvious that there are two kinds of Romanowsky stains. The one type, made by polychroming methylene blue with alkali and then precipitating eosinates of substances present in the polychromed solution, is inconstant in composition, varies considerably in dye concentration from one batch to the next, and in addition contains some substances that are useless in staining. Such stains include Leishman's (1901) and Wright's (1902, 1910). They are unscientific because the decomposition of methylene blue by alkali in the polychroming process cannot be accurately controlled. (Compare Scott and French, 1924.) Difficulties in their routine use are commonly experienced, and consist of obscuring precipitates, impossibility of obtaining uniform results on successive slides, indistinctly demonstrated granules, and hazy cell contours.

The second type is weighed out with those purified chemicals which have been demonstrated to be the active constituents formed by polychroming methylene blue solutions (Giemsa, 1902a, 1902b, 1904; Michaelis, 1901a; MacNeal, 1906, 1907, 1922, 1925). They, therefore, allow controlled variation. These stains are only two in number: Giemsa's and MacNeal's. The May-Grünwald-Giemsa technic adds no new dyes, and is useful mainly because of the type of fixation (May, 1906). It will be discussed in a subsequent paper.

Yet even these two scientific stains offer possibilities for fundamental improvement. There is good reason for doubting the correctness of the formula given by Giemsa for his stain. And to both of these more rational technics may be raised the objection of variation each time that they are employed. Wright's, Giemsa's, and MacNeal's stains require dilution with water before use. Except in Giemsa's method, this procedure is done by estimation without actual measurements, and the resulting solutions, therefore, fluctuate notably in dye concentration and solvent composition. On the other hand, while Giemsa's stain is quantitatively more accurate, it requires a much longer time for action than do the others.

With the utilization of current knowledge of the chemistry of the dyes necessary for Romanowsky staining, it has been possible in the present study to prepare a very simple solution which has definite advantages over all stains heretofore advocated for blood smears. In its use there is but one variable factor—time of staining. In addition, this same unmodified stain demonstrates megakaryocyte and platelet granules in tissues and acts extremely rapidly on frozen sections.

Thus, by appropriate treatment before and after staining, a single stain can be made to serve three distinct purposes. Being entirely synthetic, this solution is controllable and numerous changes to suit individual preferences become possible. Because a single solution is used, the concentration of the various dyes and solvents can be maintained at a constant value. Then, experiments in which constituents are varied one by one, permit for the first time elucidation of the rôle of that particular substance in Romanowsky staining.

In the development of the stain presented here, 216 staining experiments were performed. An analysis of these, with the principles involved, will follow in another paper. At present there will be given merely the final formula and its methods of use, without going into the lengthy explanations necessary for guiding the user in preparing the multiplicity of variations which are possible and indeed may serve better for a particular function.

The Stain

Formula

Solution I (Aqueous)

Dyes			Solvent	
Methylene blue, U. S. P. med.	88%	0.0650 g.	Glycerin, C. P.	5.0 cc.
Methylene azure A	80%	0.0100 g.	CH_3OH, C. P.	5.0 cc.
			Distilled water	25.0 cc.
			Buffer pH 6.9	15.0 cc.
				50.0 cc.

SOLUTION II (NON-AQUEOUS)

DYES			SOLVENT	
Methylene violet (Bernthsen)	85%.....	0.0130 g.	Glycerin, C. P.....	5.0 cc.
Eosin, yel., w. s.	92%.....	0.0450 g.	CH_3OH, C. P.....	10.0 cc
			Acetone, C. P.....	35.0 cc.
				50.0 cc.

The dyes used thus far have been manufactured by the National Aniline and Chemical Company and certified for blood stains by the Commission on Standardization of Biological Stains. An analytical chemical balance must be used for weighing. A few hours elapse before complete solution results. Solution I is slightly oversaturated with methylene azure A and may be filtered on the second day when complete saturation has occurred, altho I do not consider it necessary to do so[2]. These solutions must be kept in tightly stoppered bottles, which are absolutely clean and have ground glass stoppers. Slight variations in the purity of the dyes should be allowed for on the basis of the percentage of purity given.

The buffer solution is prepared from two standard buffers, a primary and a secondary:

Primary..... 9.078 g. KH_2PO_4 per liter

Secondary..... 11.876 g. $Na_2HPO_4 \cdot 2\ H_2O$ per liter

These are preserved with thymol crystals. The secondary salt, as usually purchased, contains 12 H_2O instead of 2 H_2O. It should, therefore, be exposed to the atmosphere for two weeks before use, to decrease the number of molecules of water of crystallization (Scott and French, 1924). A pH of about 6.9 is obtained by mixing 60 cc. of the secondary salt with 40 cc. of the primary. Checks by the quinhydrone electrode method demonstrate that the desired pH is reached by the foregoing procedure, altho a pH closer to 7.0 is indicated by pH tables.

To obtain the stain, equal volumes of the blue Solution I and the red-purple Solution II are measured out with a graduate and poured into a dropping bottle. Pipets are unnecessary if suitable graduates for a desired volume are available. A quick shake of these two solutions now results in the final stain, which looks like a diluted Wright's stain. The whole procedure here described requires but a minute, and the single staining solution thus obtained has been used with success for a period of eight months. It is not recommended, however, to prepare large quantities of this final solution because the eosinates of the other dyes present gradually precipitate. The so-

[2]Subsequent experiments with another batch of Azure A (NAz-5; the first lot having been NAz-2) indicate that the slight precipitate seen is due to the interaction of impurities in the dye rather than to oversaturation.

lution, therefore, becomes weaker very slowly until the staining time has to be increased as much as 100% after a lapse of eight and one-half months. No ripening is necessary at any stage. The amount of stain to be prepared for daily usage is easily calculated on the basis that a slide requires almost two cubic centimeters of stain to flood it. It must be emphasized that the formula given is not immutable, but that almost any of its components may be changed with good results.

Technic

FOR BLOOD SMEARS

1. Dry the smear in air.
2. Fix in methyl alcohol for one-half to one minute.
3. Dry.
4. Flood slide with stain for 5 to 8 minutes. (Speed of evaporation and depth of color desired determine the time.)
5. Wash off stain with stream of distilled water from wash bottle.
6. Dry.
7. Mount, if desired, in neutralized xylol-dammar or any other desirable medium.

FOR SECTIONS

a. Paraffin sections

(The figures represent successive Coplin jars, contents 60 cc.)

1. Xylol.
2. Descending alcohols (70% alcohol with iodine if necessary).
3. Distilled water.
4. Flood with stain for 9 to 10 minutes.
5. Rinse in distilled water (change often).
6. Wash in distilled water acidified with acetic acid, 15 drops (0.75 cc.) of 1% solution to 60 cc. of water.
7. Wash in distilled water to stop action of acid and allow examination under the microscope.
8. Wipe off excess water and rinse in C. P. acetone to which has been added, for every 60 cc., 10 drops (0.5 cc.) of 1% acetic acid and also 0.01 cc. of 5% eosin in CH_3OH (or 0.0005 g.).
9. Rinse in C. P. normal butyl alcohol to which has been added, for every 60 cc., 0.02 cc. of 5% eosin in CH_3OH (or 0.001 g.).
10. Rinse in neutralized xylol. Examine under microscope for proper differentiation. If satisfactory, go on with (11). If not, go back through (9), (8), and even (6) if necessary. Then as above again.
11. Three changes of neutralized xylol (change often).
12. Mount in neutralized xylol-dammar and keep from light.

b. Frozen sections

1. Blot to slide.
2. Then as in (4) above.
3. For speed, one may stain only 2 to 3 minutes and thereafter examine in water, without differentiation. Staining over 5 minutes is rarely required on unfixed sections. Only one

rinse in xylol is necessary for slides not to be kept permanently, and (6) above may be omitted.

Discussion

The solvent may seem to be unnecessarily complicated but definite reasons exist not only for the presence of each component, but also for the amount of that component. Acetone, contrary to general opinion is not detrimental for blood staining. It is, indeed, a very useful substance in the technic here presented.

When, for reasons of economy, the slide is not flooded with stain, the extremely volatile acetone evaporates off more rapidly in those spots where the layer of stain is thinnest and thus facilitates the formation of precipitates which, if sucked down to the level of the slide by the evaporation of the water also, obscure the cells. The only variable in this technic is the time factor. The warmer the day and the more air currents there are, the more rapidly staining occurs. An easy way to determine a suitable time for staining is by the formation of a metallic sheen of uniform density over almost the entire surface of the stain. If the stain is washed off immediately when this condition is apparent, absolutely uniform results are obtained on scores of successive slides, with all similar cellular elements the same depth of color.

For the preparation of slides for class use, or for the busy hospital technician, this method offers many advantages. Predominating are the simplicity of the method, its constancy, and its ability to reproduce the same structure in the same color. Changes seen in various preparations can definitely be attributed to the cells and not blamed on variations in the stain. For laboratories, the cheapness of the method will be inviting.

Since the colors are similar to those in good preparations made with Wright's stain, nothing new need be learned for identification of the cells. All the granules are clearly demonstrated except the basophilic ones, which seem to be washed away to a large extent by this technic. Especially easy to bring out, however, are the granules of monocytes and lymphocytes.

For frozen sections, this technic appears to save valuable time in making rush diagnoses of tumors when the patient is under an anesthetic. In a total technic time of five minutes, preparations matching hematoxylin and eosin in differentiating ability can be prepared simply and constantly. Or if such slides are to be compared with polychromed methylene blue stains, then staining for two minutes and examination in water will reveal far more detail than do the methylene blue solutions.

On hemopoietic tissues fixed in Downey's fluid, megakaryocyte and platelet granules are stained brilliantly. Interesting and important studies in this respect have already been made and will be reported later. Formalin-fixed material is not as good as that fixed in Downey's fluid, but, nevertheless, will stain well. Thus, material that has been so treated, as is almost routine in pathological laboratories, and then later is discovered to be of hematological importance, can be studied to advantage.

Material fixed in Helly's or Maximow's fluids has not been made to display megakaryocyte granules well as yet, but results similar to those following the azure-II-eosin technic are obtained. The present stain, however, is exceedingly faster.

For malaria this technic yields preparations which are so clear that oil immersion magnifications are not essential. In leukemias, the various types of granules are stained with textbook clearness even when Wright's stain fails to demonstrate them.

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MICRO-MANIPULATION OF CELLS IN MAMMALS

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ABSTRACT.—**An arrangement of apparatus for applying micro-manipulative procedures to cells in living mammals is described. It was found satisfactory for manipulation of pericapillary cells and capillary endothelium in the greater omentum of the cat.**

An animal board, a Bausch and Lomb triple purpose micro-projector and a double Fitz micro-manipulator were mounted on a spring platform. The micro-needles were drawn from Pyrex rods by hand. The stage of the microscope was modified to protect the condenser from fluids. Warm saline solution was carried to the exposed omentum thru flexible rubber tubing.

The use of a micro-dissection chamber which immobilized the part of the omentum under manipulation is explained. The construction of this chamber is shown by diagrams. A camera support was bolted to the base of the micro-manipulator.

The arrangement of apparatus is shown by a photograph.

The successful application of micro-manipulative procedures to the cells of mammals requires the mounting of the micro-manipulator, the microscope lamp and the animal board on a common base, and immobilization of the field of manipulation.

The arrangement of apparatus described here was found satisfactory by Rogers[1] for micro-manipulative studies on the pericapillary cells and capillary endothelium in the greater omentum of the cat. Any of the usual laboratory animals could be handled with this equipment.

The micro-manipulator, the lamp and animal board were mounted on a stout wooden platform (22 inches by 30 inches) supported by springs. Excessive movement was prevented by blocks of sponge rubber placed between the bottom of the platform and the table. Manipulation and photomicrography could be done at magnifications up to 1350 diameters without interference by movements caused by vibration of the building.

The animal board, made of wood, was supported at the ends by bolts and wing nuts which permitted easy adjustment to any desired position. The cat, anaesthetized with sodium diethylbarbiturate, was placed on its side on the board and tied down so that its mid-ventral line was close to that edge of the board nearest the micro-

[1]Rogers, J. B. Observations in vivo on the capillaries in the greater omentum of cats (abstract). Anat. Rec., **61**, Suppl., 42. 1935.

scope stage. Numerous strings were attached to the board so that the animal could be held securely in position without interfering with the micro-manipulator controls. The board was high enough so that the microscope lamp could be placed beneath it.

The source of light was the illuminating element of the Bausch and Lomb triple purpose micro-projector. This was mounted on a low base and placed beneath the animal board. An additional converging doublet of approximately 30 mm. focus was mounted between the light source and the mirror, producing a 10 mm. circle of light on the concave mirror. For visual observation a plate of blue glass was placed in front of the lamp. This source of light, together with a condenser whose focal point was 10 mm. above the stage of the microscope, was used.

A double micro-manipulator of the type designed by Fitz[2] was used.

The shaft of the micro-needle was made by drawing out a Pyrex rod, 6 mm. in diameter, to a diameter of 1 mm. This 1 mm. rod was then drawn at one end to form a shaftlet 2 mm. long, tapering down to 30 microns in diameter. This shaftlet was then bent to an angle of 60 degrees and a micro-tip formed in a micro-flame. The 1 mm. shaft was cut to a length of 60 mm. and mounted in the tool tip with beeswax.

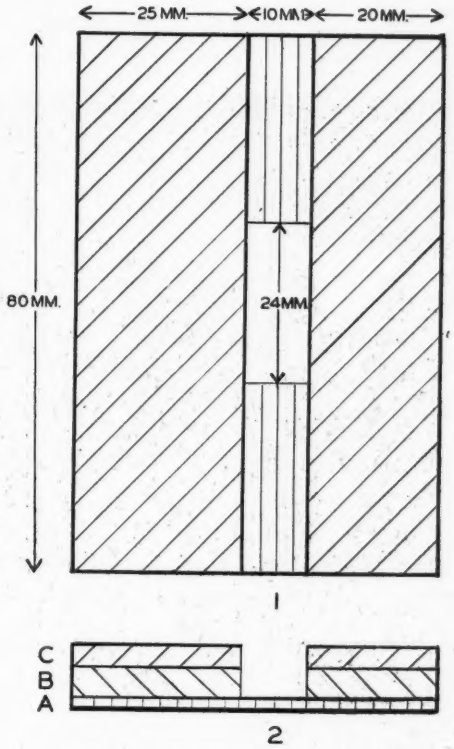
The stage of the microscope was modified to protect the condenser and the base of the micro-manipulator from saline solution which irrigated the preparation. The condenser aperture was covered with a cover slip (40 mm. by 60 mm.) cemented to the stage with rubber cement. Excess of fluid was collected in a lead trough attached to the stage, and drained away. The lead trough was bolted to the lower surface of the stage so that it did not interfere with the movements of the micro-manipulators.

A micro-dissection chamber, to which the omentum was pinned, was used. This chamber was heavy enough to immobilize the part of the omentum under manipulation. This chamber was made by casting two 5 mm. lead plates, with a 10 mm. space between them, on an aluminum base 1 mm. thick, with a central opening. Sheet cork for pinning the omentum was cemented to the upper surface of each lead plate. The dimensions of the chamber are given in Figures 1 and 2.

In use the chamber was placed on the stage of the microscope so that light was transmitted up thru the central aperture of the base. The micro-needles, one from each side, were brought in thru the space between the lead plates and the tips centered. Then a mid-ventral incision was made in the anaesthetized animal and the greater

[2]Fitz, G. W. A new micro-manipulator. Science, 73, No. 1881, 72. 1931

omentum pulled out. A fold of omentum which consisted of all layers of the omental bursa was gently stretched over the top of the chamber and pinned to the cork. It was essential that the omentum between the chamber and the animal was not under tension to avoid trans-



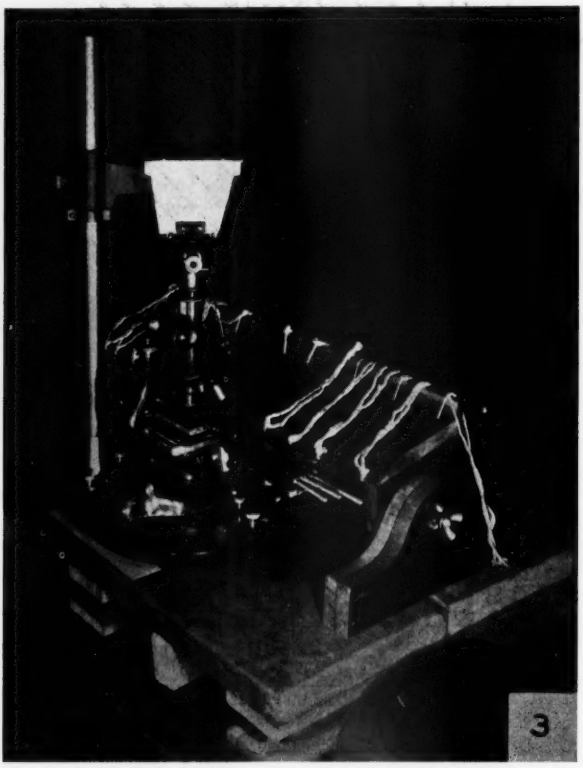
1. Chamber viewed from above giving dimensions. The aluminum sheet has a central opening for the transmission of light.

2. Sectional view of chamber.
   A—aluminum 1 mm. in thickness.
   B—lead 5 mm. in thickness.
   C—cork 3 mm. in thickness.

mission of movements of the intestines and body wall. A cover slip (22 mm. by 22 mm.) was placed on the upper surface of the omentum. Arteries which pulsated and thus would move the cover slip were avoided. The preparation was observed and the micro-needles raised until brought in contact with the cell to be studied. It usually was more satisfactory to bring the micro-needles up thru fenestrae in the

omentum and work on cells in the layer which was in contact with the cover slip. Manipulation was done under a magnification of 600 diameters (15× compensating ocular and 4 mm. objective) and 1350 diameters (15× compensating ocular and 2 mm. oil immersion objective).

Photographic records of selected fields were made with a camera, the support of which was bolted to the base of the micro-manipu-



3. Photograph of apparatus on the spring platform.

lator. The camera used was a Bausch and Lomb, model K, modified to use 6.5 cm. by 9 cm. plates.

The exposed omentum was irrigated continuously with warmed saline solution thru flexible rubber tubing. No movement was transmitted to the preparation thru the tubing.

The arrangement of spring platform, animal board, micro-manipulator, micro-dissection chamber and camera mounting is shown in Figure 3.

THE STRIP METHOD FOR COUNTING NERVE FIBERS OR OTHER MICROSCOPIC UNITS

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Abstract.—**A comparison of complete counts of nerve fibers by the strip method with the photographic method showed a minimum difference of 0.3% and a maximum of 2.9%. The preparations counted were selected for clarity and discreteness of fibers to avoid introducing an error (ambiguity of fibers) which is not pertinent to any particular method of counting. Agreement of counts with estimates indicated the feasibility of estimates when the greatest accuracy is not required.**

Three methods applicable to the enumeration of nerve cells and fibers, and other histological units, are found in the literature: the ocular net, the photographic, and the strip method.

An ocular with a single ruled square was used in 1880 by Salzer for counting fibers in the optic nerve. The squared ocular net was used by Lewin and Gaule (1896), Dunn (1902), Duncan (1930), and Ono (1931). Various forms and uses of nets have been described also by Bazin (1919). Hardesty (1898) seems to have been the first to introduce the photographic method of counting. In this, photomicrographs are used as maps and should be of sufficiently high magnification to give enough room to record as dots all of the nerve fibers seen under the highest magnification of visual observation. The fibers are checked off with colored ink as counted (preferably counted with a hand tally) and positions of fibers not resolved on the photomicrograph indicated by dots. If the entire cross section of a nerve is counted, the method's accuracy is limited only by the care with which it is done and the ambiguity of what are fibers. If only sample areas are counted, the accuracy depends upon the uniformity of distribution of fibers and the accuracy of measurement of counted and of the total area of the cross section as well as ambiguity of fibers. This method was used by Duncan (1930), Hardesty (1898), Dale (1900), Hatai (1902), and also in our laboratories.

The strip method was used by Davenport and Bothe (1934) to enumerate the cells in large dorsal root ganglia where the cross section was too large to be covered by a single field of the microscope. The determination of its limits of accuracy when applied to counting nerve fibers has been the object of the present investigation.

[1]Contribution No. 222.

An ocular is provided with two parallel lines by cementing two pieces of cover glass to the diaphragm. Smooth, straight edges are selected and the cover glass cut down with a sharp diamond to fit the diaphragm of the ocular as shown in Fig. 1. If no smooth, straight edges can be found among cover glasses available, they may be secured by polishing an edge with levigated alumina against a piece of plate glass. The abrasive is made into a paste with water, applied to the plate glass, and the edge of the cover glass rubbed gently in a direction parallel to its edge. If extraneous grit be avoided, a smooth edge can be obtained.

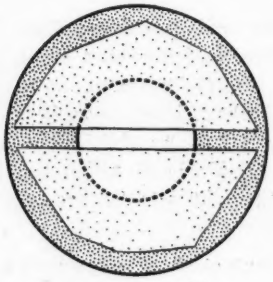


FIG. 1. PIECES OF COVER GLASS CEMENTED TO OCULAR DIAPHRAGM

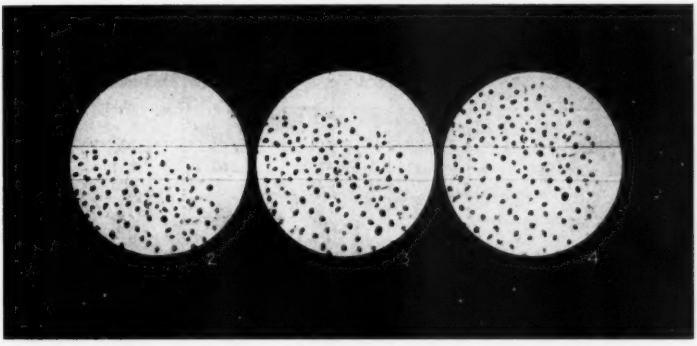
The diaphragm should be in focus and the pieces of cover glass cleaned and fastened on the eye-lens side as follows: remove the lenses and put two or three very small bits of beeswax on the diaphragm under each piece of cover glass. Heat the ocular tube with a flame until the wax melts. Align the straight edges to parallelism while the wax is fluid.

The width between the lines should vary according to the density of distribution of units to be counted. A width of 4 mm., used in conjunction with a 20× objective, has been very satisfactory for counting nucleoli of ganglion cells, while a 1 mm. width was used with an oil immersion objective when small, closely packed nerve fibers were counted. The distance between the lines should be narrow enough to permit the eye to follow the detail of the field between them. The greater the number of units in a given space to be counted, the narrower should be the space between the lines.

The actual counting is done by setting the upper line in the ocular so that it lies just above the edge of the section whose cells or fibers are to be enumerated (Fig. 2), and adjusting the ocular until the movement of the mechanical stage is parallel to the lines. One then watches the center of the field and moves the section slowly across while checking off each unit counted with a hand tally. Every unit (fiber or nucleolus) which is in contact with the lower line is *counted*. The stage is then moved vertically until the exact position on the section which was occupied by the lower line is occupied by the upper line in the new position, and the next strip across the field counted. Units in contact with the upper line are *not counted*. Figures 2, 3,

and 4 show progressive positions of landmarks as they would appear in three settings. This method of progressing from strip to strip is similar to Duncan's method from square to square in a ruled ocular, and identical with Jones' (1935) use of a ruled ocular to delineate strips.

The accuracy of the method was tested by comparing counts obtained by it with counts made from photographs of cross sections of nerves. Both myelin sheaths (osmic) and axis cylinders (silver) were counted. Table 1 shows how the counts compared. The maximum difference between any two counts was 2.9%. Other compari-



FIGS. 2–4. PROGRESSIVE POSITIONS OF LANDMARKS AS THEY APPEAR IN THE PROCESS OF COUNTING A MICROSCOPIC FIELD

sons were made between estimates made by counting the fibers in sample areas on photographs of cross sections of nerves and computing the total number by means of the proportion:

Area counted : Total area :: Number counted : Total number,

and counts by the above described strip method. The exact number of fibers in these specimens is not known but the agreement of the two methods within a maximum difference of 7.0% indicates the limitation of accuracy.

The possibility of estimating the number of nerve fibers in a cross section of a nerve trunk by counting only 20 to 25% of the total number was considered also. Since moving the section vertically the exact distance between the lines in the ocular provides the means for determining the number of strips contained in the entire section, the counting of every fifth strip, every tenth, or two adjacent strips in ten should give a close approximation to the total number of fibers. A few results (last column, Table 1) indicate that such estimates are likely to be somewhat erratic, but agree fairly well with the total

counts. A nerve whose cross section is circular and whose fibers are distributed uniformly can be estimated roughly by determining the number of strips, counting the middle one, and multiplying: No. of strips × No. of fibers counted × .785 = Total No. of fibers.

From the above results and others previously reported (Davenport and Bothe, 1934), we conclude that there are two sources of error which are potentially greater than any inherent in the method itself. These are the interpretation of unmyelinated fibers and the correction which has to be made for cut nucleoli or multiple nucleoli in ganglion cells. If one avoids mistaking stained granules in nuclei of endoneurial tissue for unmyelinated fibers, and discards preparations in which the unmyelinated fibers are either badly clumped or poorly stained, much of the former error can be avoided. The correction for any overcounting of nucleoli can be found by following several hundred cells serially and noting the percentage of double nucleoli and parts which occur in two sections of the same cell.

TABLE 1. COMPARISONS OF COUNTS AND ESTIMATES OBTAINED BY THE STRIP METHOD WITH THOSE FROM PHOTOGRAPHS

Nerve	Stain	Number of nerve fibers			
		From photograph	Strips: by Barnes	Strips: by Davenport	2 strips in 10
		Complete counts			*Estimates*
Cat Cc 1, part of roots	Os	2,240	2,219 2,220	2,226 2,234 2,228	
Rabbit, phrenic	Ag	821	824	827 837	
Cat, saphenous	Os	2,289	2,249	2,264	
Cat, phrenic	Os Ag	755 761	745 751	737 768 768	
Monkey L7, ventral root	Os		5,404	5,267	
Cat vagus, cardiac branch	Ag	3,668	3,563	3,686	3,448
		Estimates			
Man T4, dorsal root	Os Ag	10,000 20,500	9,528	9,441 20,856	9,240 22,049
" " , ventral root	Os	7,100	6,670	6,602	6,728
Man C6, ventral root	Ag	12,500		12,574	12,103

CAUTIONS

A record should be kept of the number of strips counted, when counting nerve fibers, and a sketch of the location made if the count is interrupted. Losing the place necessitates starting over again.

A mechanical stage which operates smoothly and does not slip or drift is essential.

The edges of the cover slips in the ocular should be fine and straight. Coarse lines may obscure small fibers.

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LABORATORY HINTS FROM THE LITERATURE

Under this heading are given interesting new methods in microscopy that have been noticed in other publications. The readers' assistance is urged in calling the editor's attention to any outstanding methods in this field that are overlooked.

ABET, H. Zur Technik der Geisseldarstellung nach Gray. Zentbl. Bakt., I Abt. Orig., **133,** 465–7. 1935.

For consistent results in staining the flagella of the genera *Salmonella* and *Proteus*, Gray's flagella stain has been modified in the following details:

(1) Preparation of slides: Glass slides must be new or free from blemishes; boil and stir them ½ hour in a beaker with glass beads on the bottom and a cleaning solution of a 5% $K_2Cr_2O_7$ plus 5% commercial H_2SO_4; rinse in 60% NaOH, in 5% commercial HCl, in distilled water. Store slides in equal parts of 1.5N NH_4OH and absolute alcohol; for use, rinse in absolute alcohol, pass 20 to 30 times thru the Bunsen flame and cool in thermostat. Transfer the culture thru 4 successive daily transfers from the condensation water of meat extract peptone agar. From the 4th tube, 8 hours old, remove 2 loopfuls of condensation water and suspend in 2 to 3 cc. distilled water; incubate 2 hours at room temperature. Transfer 3 loopfuls to the end of a lukewarm glass slide and by careful tilting run down the drop to a broad area on the glass. Dry in thermostat, fix in the flame by describing twice in 1 second a circle 30 cm. in diameter.

(2) Mordanting and staining: Gray's mordant consists of the 2 following solutions: (a) saturated potassium alum, 5 cc; saturated sublimate solution, 2 cc; 20% tannic acid solution, 2 cc.; (b) saturated basic fuchsin alcohol solution, 0.4 cc. Mix and filter. Sink slides standing on end into the mordant for 7 to 10 minutes. Wash in strong stream of water and stain 5 minutes in Ziehl's carbol-fuchsin by resting the slides face down on 2 glass rods on the bottom of a Petri dish.

HANSBURG, L. A new staining method for structures of the spinal cord. Science, **81,** 364–5. 1935.

Disadvantages in staining cytons and Nissl bodies of the spinal cord may be overcome by employing dyes that contain greater amounts of methylene violet than one can obtain from polychromed methylene blue. For selectivity and permanency the following procedure is suggested: Fix material in 10% acid-free formaldehyde and cut in paraffin at 10μ. Bring down to distilled water and flood 2 minutes with a mixture of 5 parts of Wright's stain solution in 95% denatured ethyl alcohol and 1 part Giemsa standard solution. Dilute the dye with an equal amount of distilled water for 2 minutes, immerse slides in fresh distilled water for 1 minute. Pass immediately into 80% alcohol for 15 seconds; dehydrate rapidly in 95% and absolute alcohol. Clear and mount in neutral balsam. Cytons and Nissl granules appear deep blue; nuclei of blood vessel structures and neuroglia, light blue; elastic fibers of blood vessels, deep blue; erythrocytes, pink; and neuroglia fibers, light red. The proportion of Giemsa solution regulates the intensity of cyton stain. (No information as to source of stains is given.)

MATSUO, I. Biologische Untersuchungen über Farbstoffe. 1. Band. 494 pp. Kyoto, 1934. (Abstr. in Zentbl. Bakt., I Abt. Ref., **117,** 406. 1935.)

This is the first volume of a comprehensive treatise on dyestuffs in biological research. It covers the result of systematic experimentation with 300 dyes as carried on by the author and 46 students over a period of 9 years. Most of the findings have been previously published elsewhere. The first section deals with the elimination and resorption of the dyestuffs in general; the other 3 sections discuss their physico-chemistry and pharmacology, especially with reference to excretion thru liver and kidney.

WEICHHERZ, E. Die Kahnsche Reaktion mit Färbung nach Hecht. Čas. lék. čes. 1934, 1096. (Abs. in Zentbl. Bakt., I Ref., **117,** 438. 1935.)

The reading of the Kahn reaction by means of a magnifier or the agglutinoscope is not sufficiently precise. The technic suggested here is a modification of Hecht's sudan method. Add a knifetip portion of Sudan R III (I. G. Farben) to 100 cc. of the Kahn extract; filter after 25 hours. According to the titer, add the required amount of saline and incubate 10 minutes at room temperature. Pipet the extract into the serum, shake 5 minutes, add 0.5 cc. saline to each tube and centrifuge 10 minutes at 2000 r.p.m. A dot of red precipitate, after decantation, indicates a positive reaction.

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the July number of this journal.

STAINS CERTIFIED JUNE 1, 1935 TO AUG. 31, 1935*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Crystal violet	CC 7	93%	As bacteriological, histological and cytological stain	June 3, 1935
Hematoxylin	FH 12	——	As histological and cytological stain	June 26, 1935
Tetrachrome stain	NMn 7	——	As blood stain	July 5, 1935
Neutral red	NX 6	61%	As histological and vital blood stain	July 12, 1935
Eosin Y	LE 9	92%	In histology and as constituent of blood stains	Aug. 6, 1935
Malachite green	LMg 2	97%	As histological and bacteriological stain	Aug. 6, 1935
Pyronin	NP 5	56%	As constituent of Pappenheim stain	Aug. 6, 1935
Eosin Y	NE 11	89%	In histology and as constituent of blood stains	Aug. 9, 1935

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

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